Enzo

Hepatitis B Microplate Hybridization Assay

Cat. No. 46350

•For Research Use Only

INTENDED USE

The ENZO Hepatitis B Microplate Hybridization Assay Kit provides materials for the detection of hepatitis B core region DNA sequences in a microplate assay using a biotin-based detection system.

SUMMARY, EXPLANATION AND PRINCIPLE

The ENZO Hepatitis B Microplate Hybridization Assay is a non-radioactive, colorimetric hybridization procedure performed in a microwell format. Hepatitis B DNA can be assayed indirectly in procedures employing target amplification, or it can be assayed directly if there is sufficient target DNA present. The detection procedure has been developed for use with a streptavidin-horseradish peroxidase complex to visualize the presence of biotin-labeled probes.

The ENZO non-radioactive procedure involves pretreatment of the sample to denature the DNA, followed by hybridization of the DNA to a well-bound capture probe. The captured hepatitis B DNA is then hybridized to a signal probe. The resulting hybrid is reacted with a biotin-labeled oligomer, and the biotin is detected using a streptavidin-horseradish peroxidase detection system. A positive reaction is indicated by the appearance of bright yellow color which can be measured by a microplate reader. Using this format, 10^7 to 10^8 copies of target sequences can be detected.

STORAGE

Store the kit at 2°-8°C. DO NOT FREEZE. When used and stored as directed, the kit is stable until the expiration date indicated on the box.

REAGENTS AND MATERIALS PROVIDED

The ENZO Hepatitis B Microplate Hybridization Assay Kit provides reagents for testing 96 samples in a microwell strip format.

Vial 1 **Denaturation Reagent, 3 ml**
Dilute alkaline solution containing indicator

U.S. Patent Nos. 4,711,955, 4,994,373, and 5,328,824, EP 0 063 879 B1, EP 0 117 40 B1 and Patents Pending

Vial 2 **Hybridization Buffer, 10 ml**
Buffered sodium chloride/EDTA containing formamide and hybridization enhancers

Vial 3 **HBV Signal Probe, 6 ml**
Modified hepatitis B-specific DNA probe in buffered sodium chloride/EDTA containing formamide, hybridization enhancers and indicator

Vial 4 **Linker, 6 ml**
Modified poly-dA in buffered sodium chloride/sodium citrate containing detergent

Vial 5 **20X Rinse Buffer, 25 ml**
Buffered sodium chloride/sodium citrate containing detergent

Vial 6a **10X Detection Reagent, 1.0 ml**
Streptavidin-horseradish peroxidase complex in buffered sodium chloride, stabilizer and detergent

Vial 6b **Detection Buffer, 10 ml**
Buffered sodium chloride/EDTA containing stabilizer and detergent

Vial 7a **Chromogen Reagent, 1.5 ml**
5 mg/ml tetramethylbenzidine (TMB) in solvent

Vial 7b **Reaction Buffer/Substrate Reagent, 15 ml**
Dilute hydrogen peroxide in citrate phosphate buffer

Vial 8 **Stop Solution, 12 ml**
Dilute acid solution

Vial 9 **HBV Positive Control, 100 µl**
Plasmid DNA carrying hepatitis B DNA sequences

Precoated Microwells, 6 microwell strips (2 x 8) in a strip holder, 96 wells
Microwells coated with hepatitis B-specific capture probe

Plate Sealer, 1

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate shaker
- Precision pipets capable of delivering volumes of 5 µl to 1 ml
- Polypropylene microtubes
- Microplate reader (*optional*)
- Sterile distilled water
- TE buffer (10mM Tris, pH 8.0, 1mM EDTA), for use when diluting samples prior to assay

WARNINGS

- **For RESEARCH use only! Not to be used for *in vitro* diagnostic purposes.**
- Read all instructions prior to performing this assay.
- Wear disposable gloves while handling kit reagents and specimens. Wash hands thoroughly after handling.
- Do not smoke, eat, drink or apply cosmetics in areas in which specimens or kit reagents are handled.
- Do not pipet by mouth.

- Use a separate disposable pipet or pipet tip for each transfer of sample to avoid cross-contamination.
- Ensure that all test samples and controls are subjected to the same processing and incubation times. Once the assay has been started, unless otherwise indicated, all subsequent steps should be completed without interruption and within the time limits recommended by the procedure.
- **Chemical Hazards.** The following reagents should be handled with care as detailed below.

  Denaturation Reagent (Vial 1) contains sodium hydroxide which is poisonous and can cause severe burns. Do not ingest or breathe vapor and avoid contact with skin, eyes or clothing. Wash after handling.

  Hybridization Buffer (Vial 2) and Signal Probe (Vial 3) contain formamide which is a teratogen and an irritant. Skin contact should be avoided. Specifically, pregnant workers should avoid any exposure.If skin contact is made, wash thoroughly with soap and water.

  Chromogen Reagent (Vial 7a) contains dimethylformamide. Use glass and/or polypropylene pipets and containers when diluting. It can cause skin irritation. If skin contact is made, wash thoroughly with soap and water.

  Stop Solution (Vial 8) contains dilute sulfuric acid which i. poisonous and can cause severe burns. Do not ingest or breathe vapor and avoid contact with skin, eyes or clothing. Wash after handling.

ASSAY CONSIDERATIONS

- The ENZO Hepatitis B Microplate Hybridization Assay Kit contains sufficient reagents and materials to analyze 96 samples, including positive and negative controls.
- If the test is to be performed on diluted material, TE buffer (10mM Tris, pH 8.0, 1mM EDTA) should be used as the diluent.
- Each time an assay is run, include appropriate positive and negative controls in parallel with the samples to be analyzed.

PREPARATION OF REAGENTS

1X Rinse Buffer: Dilute the 20X Rinse Buffer (Vial 5) 1:20 in sterile distilled water. Once diluted, the buffer must be kept at 2° - 8°C when not in use and must be used within one week of preparation.

1X Detection Reagent: Dilute the 10X Detection Reagent (Vial 6a) 1:10 in Detection Buffer (Vial 6b). Gently mix. Use within two hours.

Chromogen/Substrate Mixture: Using glass or polypropylene pipets and mixing container, prepare Chromogen/Substrate Mixture by adding 100 µl of Chromogen Reagent (Vial 7a) per 1 ml of Reaction Buffer/Substrate Reagent (Vial 7b). Mix well and keep in the dark. This solution must be prepared fresh for each test run.

SAMPLE PREPARATION

NOTE: Warm all reagents and test components to room temperature prior to beginning the assay.

STEP 1: Pipet 30 µl of **Denaturation Reagent** (Vial 1) into each of a sufficient number of polypropylene microtubes to accommodate the number of samples and controls to be assayed.

STEP 2: To the tubes prepared in step 1, add 10 µl of each sample to be tested, including a **Positive Control** (Vial 9) and a negative control (TE buffer).

STEP 3: Incubate the tubes (samples and controls) at room temperature for 15 minutes to denature the target nucleic acid sequences.

HYBRIDIZATION/DETECTION PROCEDURE

NOTE: a. *All steps are performed at room temperature. Room temperature for the purposes of this assay is defined as 23-27°C. The assay may be performed at fixed temperatures within this range. As in any temperature-dependent reaction, the quantitative values obtained will depend on the temperature at which the reaction is performed.*

b. *Do not allow the wells to dry out between steps.*

c. *Secure microwell strips with strip etainer or adhesive tape.*

STEP 4: Rinse each microwell 5 times with **1X Rinse Buffer** (diluted from 20X solution, see **Preparation of Reagents**) using 200 µl each rinse. Flick the contents of the microwells into a suitable liquid waste container and blot off the residual liquid on an absorbent surface, *e.g.*, stacked paper towels, after each wash.

STEP 5: Add 80 µl of **Hybridization Buffer** (Vial 2) to each well. Then, add 20 µl **denatured samples** to the appropriate wells.

STEP 6: After adding all samples to the microwells, seal the plate/strip(s) and incubate with shaking for 120 minutes to allow hybridization of target DNA to the well-bound capture probe. The samples will turn from blue to yellow.

STEP 7: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Then, add 50 µl of **Signal Probe** (Vial 3) to each well and incubate with shaking for 15 minutes.

STEP 8: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Rinse each microwell 5 times with 200 µl of **1X Rinse Buffer**, flicking the liquid and blotting in between each wash. Then, add 50 µl of **Linker** (Vial 4) to each well and incubate with shaking for 10 minutes.

STEP 9: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Then, add 50 µl of **1X Detection Reagent** (diluted from 10X solution, see **Preparation of Reagents**) to each well and incubate with shaking for 15-20 minutes.

STEP 10: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Rinse each microwell 5 times with 200 µl of **1X Rinse Buffer**, flicking the liquid and blotting in between each wash. Then, add 100 µl of **Chromogen/Substrate Mixture** (prepared from Vials 7a and 7b, see **Preparation of Reagents**) to each well and incubate in the dark for 15 minutes. Positive samples will turn blue.

STEP 11: Stop the color reaction by adding 100 µl of **Stop Solution** (Vial 8) to each well. Positive samples will turn from blue to yellow.

INTERPRETATION OF RESULTS

- A positive result appears as a blue color which develops after addition of the Chromogen/Substrate Mixture. The blue color changes to yellow upon addition of the Stop Solution.
- Results may be quantified by reading OD at 450 nm using a microplate reader. The positive control should give an OD reading of at least 0.5 when the assay is performed at 23-24°C. When the assay is performed at higher temperatures the positive control will give a higher OD reading.

For Technical Assistance call ENZO:
Toll free from the U.S. and Canada 1-800-221-7705
From New York State: 516-694-7070
Telefax: 516-694-7501

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Enzo
Enzo Diagnostics, Inc.
Farmingdale, New York

Enzo

Hepatitis B HBs Microplate Hybridization Assay

Cat. No. 46350S

•For Research Use Only

INTENDED USE

The ENZO Hepatitis B HBs Microplate Hybridization Assay Kit provides materials for the detection of hepatitis B Surface Antigen DNA sequences in a microplate assay using a biotin-based detection system.

SUMMARY, EXPLANATION AND PRINCIPLE

The ENZO Hepatitis B HBs Microplate Hybridization Assay is a non-radioactive, colorimetric hybridization procedure performed in a microwell format. Hepatitis B DNA can be assayed indirectly in procedures employing target amplification, or it can be assayed directly if there is sufficient target DNA present. The detection procedure has been developed for use with a streptavidin-horseradish peroxidase complex to visualize the presence of biotin-labeled probes.

The ENZO non-radioactive procedure involves pretreatment of the sample to denature the DNA, followed by hybridization of the DNA to a well-bound capture probe. The captured hepatitis B DNA is then hybridized to a signal probe. The resulting hybrid is reacted with a biotin-labeled oligomer, and the biotin is detected using a streptavidin-horseradish peroxidase detection system. A positive reaction is indicated by the appearance of bright yellow color which can be measured by a microplate reader. Using this format, 10^7 to 10^8 copies of target sequences can be detected.

STORAGE

Store the kit at 2°-8°C. DO NOT FREEZE. When used and stored as directed, the kit is stable until the expiration date indicated on the box.

REAGENTS AND MATERIALS PROVIDED

The ENZO Hepatitis B HBs Microplate Hybridization Assay Kit provides reagents for testing 96 samples in a microwell strip format.

Vial 1 **Denaturation Reagent, 3 ml**
Dilute alkaline solution containing indicator

U.S. Patent Nos. 4,711,955, 4,994,373, and 5,328,824, EP 0 063 879 B1, EP 0 117 40 B1 and Patents Pending

Vial 2 **Hybridization Buffer, 10 ml**
Buffered sodium chloride/EDTA containing formamide and hybridization enhancers

Vial 3 **HBV HBs Signal Probe, 6 ml**
Modified hepatitis B-specific DNA probe in buffered sodium chloride/EDTA containing formamide, hybridization enhancers and indicator

Vial 4 **Linker, 6 ml**
Modified poly-dA in buffered sodium chloride/sodium citrate containing detergent

Vial 5 **20X Rinse Buffer, 25 ml**
Buffered sodium chloride/sodium citrate containing detergent

Vial 6a **10X Detection Reagent, 1.0 ml**
Streptavidin-horseradish peroxidase complex in buffered sodium chloride, stabilizer and detergent

Vial 6b **Detection Buffer, 10 ml**
Buffered sodium chloride/EDTA containing stabilizer and detergent

Vial 7a **Chromogen Reagent, 1.5 ml**
5 mg/ml tetramethylbenzidine (TMB) in solvent

Vial 7b **Reaction Buffer/Substrate Reagent, 15 ml**
Dilute hydrogen peroxide in citrate phosphate buffer

Vial 8 **Stop Solution, 12 ml**
Dilute acid solution

Vial 9 **HBV Positive Control, 100 µl**
Plasmid DNA carrying hepatitis B DNA sequences

Precoated Microwells, 6 microwell strips (2 x 8) in a strip holder, 96 wells
Microwells coated with hepatitis B-specific capture probe

Plate Sealer, 1

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate shaker
- Precision pipets capable of delivering volumes of 5 µl to 1 ml
- Polypropylene microtubes
- Microplate reader (*optional*)
- Sterile distilled water
- TE buffer (10mM Tris, pH 8.0, 1mM EDTA), for use when diluting samples prior to assay

WARNINGS

- **For RESEARCH use only! Not to be used for *in vitro* diagnostic purposes.**
- Read all instructions prior to performing this assay.
- Wear disposable gloves while handling kit reagents and specimens. Wash hands thoroughly after handling.
- Do not smoke, eat, drink or apply cosmetics in areas in which specimens or kit reagents are handled.
- Do not pipet by mouth.

- Use a separate disposable pipet or pipet tip for each transfer of sample to avoid cross-contamination.
- Ensure that all test samples and controls are subjected to the same processing and incubation times. Once the assay has been started, unless otherwise indicated, all subsequent steps should be completed without interruption and within the time limits recommended by the procedure.
- **Chemical Hazards.** The following reagents should be handled with care as detailed below.

  Denaturation Reagent (Vial 1) contains sodium hydroxide which is poisonous and can cause severe burns. Do not ingest or breathe vapor and avoid contact with skin, eyes or clothing. Wash after handling.

  Hybridization Buffer (Vial 2) and Signal Probe (Vial 3) contain formamide which is a teratogen and an irritant. Skin contact should be avoided. Specifically, pregnant workers should avoid any exposure.If skin contact is made, wash thoroughly with soap and water.

  Chromogen Reagent (Vial 7a) contains dimethylformamide. Use glass and/or polypropylene pipets and containers when diluting. It can cause skin irritation. If skin contact is made, wash thoroughly with soap and water.

  Stop Solution (Vial 8) contains dilute sulfuric acid which is poisonous and can cause severe burns. Do not ingest or breathe vapor and avoid contact with skin, eyes or clothing. Wash after handling.

ASSAY CONSIDERATIONS

- The ENZO Hepatitis B HBs Microplate Hybridization Assay Kit contains sufficient reagents and materials to analyze 96 samples, including positive and negative controls.
- If the test is to be performed on diluted material, TE buffer (10mM Tris, pH 8.0, 1mM EDTA) should be used as the diluent.
- Each time an assay is run, include appropriate positive and negative controls in parallel with the samples to be analyzed.

PREPARATION OF REAGENTS

1X Rinse Buffer: Dilute the 20X Rinse Buffer (Vial 5) 1:20 in sterile distilled water. Once diluted, the buffer must be kept at 2° - 8°C when not in use and must be used within one week of preparation.

1X Detection Reagent: Dilute the 10X Detection Reagent (Vial 6a) 1:10 in Detection Buffer (Vial 6b). Gently mix. Use within two hours.

Chromogen/Substrate Mixture: Using glass or polypropylene pipets and mixing container, prepare Chromogen/Substrate Mixture by adding 100 µl of Chromogen Reagent (Vial 7a) per 1 ml of Reaction Buffer/Substrate Reagent (Vial 7b). Mix well and keep in the dark. This solution must be prepared fresh for each test run.

SAMPLE PREPARATION

NOTE: Warm all reagents and test components to room temperature prior to beginning the assay.

STEP 1: Pipet 30 µl of **Denaturation Reagent** (Vial 1) into each of a sufficient number of polypropylene microtubes to accommodate the number of samples and controls to be assayed.

STEP 2: To the tubes prepared in step 1, add 10 µl of each sample to be tested, including a **Positive Control** (Vial 9) and a negative control (TE buffer) and mix.

STEP 3: Incubate the tubes (samples and controls) at room temperature for 15 minutes to denature the target nucleic acid sequences.

HYBRIDIZATION/DETECTION PROCEDURE

NOTE: a. All steps are performed at room temperature. Room temperature for the purposes of this assay is defined as 23-27°C. The assay may be performed at fixed temperatures within this range. As in any temperature-dependent reaction, the quantitative values obtained will depend on the temperature at which the reaction is performed.

b. Do not allow the wells to dry out between steps.

c. Secure microwell strips with strip etainer or adhesive tape.

STEP 4: Rinse each microwell 5 times with **1X Rinse Buffer** (diluted from 20X solution, see **Preparation of Reagents**) using 200 µl each rinse. Flick the contents of the microwells into a suitable liquid waste container and blot off the residual liquid on an absorbent surface, *e.g.*, stacked paper towels, after each wash.

STEP 5: Add 80 µl of **Hybridization Buffer** (Vial 2) to each well. Then, add 20 µl **denatured samples** to the appropriate wells.

STEP 6: After adding all samples to the microwells, seal the plate/strip(s) and incubate with shaking for 120 minutes to allow hybridization of target DNA to the well-bound capture probe. The samples will turn from blue to yellow.

STEP 7: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Then, add 50 µl of **HBV HBs Signal Probe** (Vial 3) to each well and incubate with shaking for 15 minutes.

STEP 8: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Rinse each microwell 5 times with 200 µl of **1X Rinse Buffer**, flicking the liquid and blotting in between each wash. Then, add 50 µl of **Linker** (Vial 4) to each well and incubate with shaking for 10 minutes.

STEP 9: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Then, add 50 µl of **1X Detection Reagent** (diluted from 10X solution, see **Preparation of Reagents**) to each well and incubate with shaking for 15-20 minutes.

STEP 10: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution, as in step 4. Rinse each microwell 5 times with 200 µl of **1X Rinse Buffer**, flicking the liquid and blotting in between each wash. Then, add 100 µl of **Chromogen/Substrate Mixture** (prepared from Vials 7a and 7b, see **Preparation of Reagents**) to each well and incubate in the dark for 15 minutes. Positive samples will turn blue.

STEP 11: Stop the color reaction by adding 100 µl of **Stop Solution** (Vial 8) to each well. Positive samples will turn from blue to yellow.

INTERPRETATION OF RESULTS

- A positive result appears as a blue color which develops after addition of the Chromogen/Substrate Mixture. The blue color changes to yellow upon addition of the Stop Solution.
- Results may be quantified by reading OD at 450 nm using a microplate reader. The positive control should give an OD reading of at least 0.5 when the assay is performed at 23-24°C. When the assay is performed at higher temperatures the positive control will give a higher OD reading.

For Technical Assistance call ENZO:
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From New York State: 516-694-7070
Telefax: 516-694-7501

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This Product is sold by ENZO DIAGNOSTICS, INC. for research purposes only and is not intended for diagnostic or therapeutic use. Purchase does not include any right or license to exploit this product commercially. Any commercial development of this product without the express written authorization of ENZO DIAGNOSTICS, INC. is prohibited.

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Enhanced Microplate Hybridization Assay for Hepatitis B DNA*

Cat. No. 46353

•For Research Use Only

INTENDED USE

This kit is intended for use in detection of Hepatitis B viral (HBV) DNA in human serum specimens by an Enhanced Microplate Hybridization Assay. The kit contains reagents for processing and assaying serum specimens for a combination of Hepatitis B core and surface antigen DNA sequences.

SUMMARY, EXPLANATION AND PRINCIPLE

The ENZO Enhanced Microplate Hybridization Assay for Hepatitis B DNA is a nonradioactive, colorimetric hybridization procedure performed in a microwell format on human serum specimens. Hepatitis B core and surface antigen DNA sequences can be assayed directly from serum in this procedure by first treating the serum with a Sample Digestion Reagent designed to digest serum components and to release viral DNA. The DNA is then denatured by treatment with alkali and heat. The processed material, containing denatured DNA, is hybridized to HBV capture DNA sequences fixed to a microwell surface. The captured DNA is detected by hybridization with an HBV-specific signal probe mixture. The resulting hybrid is reacted with a biotin-labeled oligomer, and the biotin is detected using a high-sensitivity streptavidin-horseradish peroxidase conjugate. A positive reaction is indicated by the appearance of bright yellow color which can be measured by a microplate reader. Using this format, approximately 10^5-10^6 copies of target Hepatitis B viral DNA sequences can be detected.

STORAGE

Store the kit at 2°-8°C. DO NOT FREEZE. When used and stored as directed, the kit is stable until the expiration date indicated on the box.

REAGENTS

The ENZO Enhanced Microplate Hybridization Assay for Hepatitis B DNA provides reagents for preparation and testing of 48 duplicate or 96 single well samples in a microwell strip format.

Vial SD **Sample Digestion Reagent, 8 ml**
Acidified aqueous detergent solution with pH indicator

Vial 1 **NaOH Denaturant, 4 ml**
3N NaOH

**U.S. Patent Nos. 4,711,955, 4,994,373, and 5,328,824, EP 0 063 879 B1, EP 0 117 440 B1, EP 0 122 614 B1, Canadian Patent Nos. 1,219,824, 1,309,672, and 1,254,525 and Patents Pending*

Vial 2 **RS Hybridization Buffer, 10 ml**
Buffered NaCl/EDTA containing formamide and hybridization enhancers

Vial 3 **HBV Signal Probe Mixture, 6 ml**
Modified Hepatitis B-specific DNA probes in buffered NaCl/EDTA containing formamide, hybridization enhancers and indicator

Vial 4 **Linker, 6 ml**
Modified poly dA in sodium chloride/sodium citrate and detergent

Vial 5 **20X Rinse Buffer, 25 ml**
Sodium chloride/sodium citrate and detergent

Vial 6a **10X HP Detection Reagent, 1 ml**
Streptavidin-horseradish peroxidase conjugate in buffered sodium chloride, stabilizer and detergent

Vial 6b **Detection Buffer, 10 ml**
Buffered NaCl/EDTA containing stabilizer and detergent

Vial 7a **Chromogen Reagent, 1.5 ml**
5 mg/ml tetramethylbenzidine (TMB) in solvent

Vial 7b **Reaction Buffer/Substrate Reagent, 15 ml**
Dilute hydrogen peroxide in citrate phosphate buffer

Vial 8 **Stop Solution, 12 ml**
Dilute acid solution

Vial 9 **Hepatitis B Positive Control, 0.5 ml**
Pre-treated plasmid DNA carrying Hepatitis B DNA sequences

Vial 10 **HBV DNA Preparation Control, 0.5 ml**
Plasmid DNA carrying HBV DNA sequences in heat inactivated calf serum

Precoated Microwells, 6 microwell strips (2 x 8) in a strip holder, 96 wells
Microwells coated with Hepatitis B-specific capture probes

Plate Sealer, 1

MATERIALS REQUIRED BUT NOT PROVIDED

- Serum specimens
- 1.5ml microcentrifuge tubes for specimen preparation
- Microplate shaker
- Precision pipets capable of delivering volumes of 5 µl to 1 ml
- Adjustable multichannel pipet
- Disposable pipet tips
- Microplate or microstrip reader with filter for 450nm
- 37°C ± 0.5°C water bath
- 65°C ± 1°C water bath
- Good quality distilled or deionized water

WARNINGS

- **For RESEARCH use only! Not to be used for *in vitro* diagnostic purposes.**
- Read all instructions prior to performing this assay.
- Ensure that all test samples and controls are subjected to the same processing and incubation times as specified in this assay protocol. Once the assay has been started, all subsequent steps should be completed without interruption and within the time limits recommended in the procedure.
- Use a safety pipetting device for all pipetting. Never pipet by mouth.
- To avoid cross-contamination of samples, use a separate disposable pipet tip for each transfer of sample and for each addition of a reagent to a series of samples.
- Wear disposable gloves while handling kit reagents and specimens. Wash hands thoroughly after handling.
- **Chemical Hazards.** The following reagents should be handled with care as detailed below.

  Sample Digestion Reagent (Vial SD) contains dilute HCl which can cause burns. Do not ingest or breathe vapor. Avoid contact with skin, eyes or clothing. Wash after handling.

  NaOH Denaturant (Vial 1) contains 3N NaOH which i poisonous and can cause severe burns. Do not ingest or breathe vapor. Avoid contact with skin, eyes or clothing. Wash after handling.

  RS Hybridization Buffer (Vial 2) and Signal Probe (Vial 3) contain formamide which is a teratogen and an irritant. Skin contact should be avoided. Specifically, pregnant workers should avoid any exposure. If skin contact is made, wash thoroughly with soap and water.

  Chromogen Reagent (Vial 7a) contains dimethylformamide (DMF). Use glass and/or polypropylene pipets and containers when diluting. DMF can cause skin irritation. If skin contact is made, wash thoroughly with soap and water.

  Stop Solution (Vial 8) contains dilute H_2SO_4 which is poisonous and can cause severe burns. Do not ingest or breathe vapor. Avoid contact with skin, eyes or clothing. Wash after handling.
- Do not substitute reagents from other manufacturers.
- Do not substitute reagents or buffers from other ENZO kits.
- Incubation times and temperatures other than those specified may give erroneous results.

PREPARATION OF REAGENTS

1X Rinse Buffer: Dilute the 20X Rinse Buffer (Vial 5) 20-fold in sterile distilled water. Once diluted, the buffer must be kept at 2°- 8°C when not in use. Use 1X Rinse Buffer within one week of preparation.

1X HP Detection Reagent: When needed for use, dilute a sufficient amount of the 10X HP Detection Reagent (Vial 6a) 10-fold, using Detection Buffer (Vial 6b). Mix gently. Prepare this diluted material fresh for each test run and use the 1X preparation within 1 hour of dilution.

Chromogen/Substrate Mixture: Using glass or polypropylene pipets and mixing container, prepare Chromogen/Substrate Mixture by adding 100 µl of Chromogen Reagent (Vial 7a) per 1 ml of Reaction Buffer/Substrate Reagent (Vial 7b). Mix well and keep in the dark. Prepare this solution fresh for each test run immediately before use.

ASSAY CONSIDERATIONS

- The ENZO Enhanced Microplate Hybridization Assay for Hepatitis B contains sufficient reagents and materials to analyze 48 samples in duplicate wells, including positive and negative controls. In order to assay 96 single well samples, use one-half the volumes indicated below in the SAMPLE PREPARATION procedures.
- Each time an assay is run, include appropriate positive and negative controls in parallel with the samples to be analyzed. Serum specimen titration standards are available separately (ENZO Cat. No. 46354).
- The Hepatitis B Positive Control (Vial 9) contains a portion of the HBV DNA genome in purified form. It is to be used as directed in the protocol (see STEP 5) as a control for the assay procedure only.
- The HBV DNA Preparation Control (Vial 10) contains HBV DNA in a recombinant plasmid (pUC19) in heat inactivated fetal calf serum. This preparation, which serves as a control for the preparation of serum specimens and as a control for the assay procedures, should be treated exactly as for serum specimens, beginning with STEP 1.

SAMPLE PREPARATION

NOTE:

ALL MIXING STEPS SHOULD BE PERFORMED BY REPEATEDLY (2-3 TIMES) INVERTING THE TUBES IN WHICH THE SAMPLES ARE PREPARED.

The digestion temperature in STEP 2 is critical and MUST be 37°C ± 0.5°C.

Following STEP 2, do not allow digested samples to stand at room temperature for longer than 5-10 minutes before adding NaOH Denaturant (VIAL 1).

Make sure that the contents of the tubes are well, but gently, mixed before incubating at 65°C in STEP 4.

STEP 1: For each sample to be assayed in **duplicate** wells, pipet **125µl of Sample Digestion Reagent (VIAL SD)** into a clean 1.5ml microcentrifuge tube. Add **125µl of sample to be assayed (or 125µl of HBV DNA Preparation Control, VIAL 10)** and **mix**. Keep all tubes at room temperature until all additions have been made.

STEP 2: Mix the contents of the tubes again, and incubate **10 minutes in a 37°C** water bath.

STEP 3: Remove tubes from the 37°C water bath and place at room temperature. Add **50µl of NaOH Denaturant (VIAL 1)** to each tube and **MIX** immediately. The mixture will turn from violet/red to blue.

STEP 4: Mix again and incubate the tubes for **10 minutes in a 65°C** water bath.

STEP 5: Remove the samples to room temperature for 5-15 minutes while preparing the Hepatitis B Positive Control (see below) and the microplate strips (see Step 6).

To prepare the Hepatitis B Positive Control for assay, pipet **125 µl** of Sample Digestion Reagent **(VIAL SD)** into a 1.5ml microcentrifuge tube. Add **50µl** of NaOH Denaturant **(VIAL 1)**. Mix the contents of the tube. Add **125µl of the Hepatitis B Positive Control (Vial 9)** and mix thoroughly, but gently. Allow this sample to stand at room temperature for 5 minutes.

HYBRIDIZATION/DETECTION PROCEDURE

NOTE:

The performance of this assay is greatly dependent upon shaking the microplate where indicated.

Do not allow samples to stand for more than 20 minutes before adding to the capture wells (STEP 7).

All of the following steps are performed at room temperatures (22°C to 27°C). The assay may be performed at a fixed temperature within this range, if desired, by using a temperature controlled microplate shaker. Because the assay is temperature dependent, absolute quantitative values will depend upon the temperature at which the assay is performed.

Do not allow the wells to dry out between steps. Keep wells filled with 200µl of 1X Rinse Buffer until ready to add reagents.

Secure microwell strips to the strip holder frame with a strip retainer or adhesive tape.

Seal unused strips with a portion of the plate sealer and store at 2°C to 8°C until needed.

Mix processed samples thoroughly, but gently, before adding 100µl portions to the capture wells (STEP 7).

STEP 6: Rinse each microwell **5 times with 1X Rinse Buffer** (see **Preparation of Reagents**) using **200 µl** for each rinse. Flick the contents of the microwells into a suitable liquid waste container and blot off the residual liquid on absorbent paper after each wash.

STEP 7: Add **80 µl of RS Hybridization Buffer (Vial 2)** to each pre-rinsed microwell. Then, mixing each sample before pipetting, add **100 µl of each treated (Steps 1-5) sample** to the appropriate wells.

STEP 8: After adding all samples to the microwells, seal the strips with the plate sealer and incubate the strips ***with shaking*** **for at least 3 hours** to allow hybridization to occur. Upon mixing, the hybridization mixture turns yellow in color.

NOTE: *Hybridization to the capture probes in the microwells can be allowed to proceed for up to 20 hours (i.e., overnight) at room temperature, with shaking.*

STEP 9: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution.
Add **50 µl of Signal Probe Mixture (Vial 3)** to each well and incubate ***with shaking*** for 15 minutes.

STEP 10: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution. **Rinse each microwell 5 times with 200 µl of 1X Rinse Buffer**, flicking the liquid and blotting in between each wash.
Add **50 µl of Linker (Vial 4)** to each well and incubate ***with shaking*** for 10 minutes.

STEP 11: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution. **Rinse each microwell 5 times with 200 µl of 1X Rinse Buffer**, flicking the liquid and blotting in between each wash.
Add 50 µl of 1X HP Detection Reagent (see **Preparation of Reagents**) to each well and incubate ***with shaking*** for 30 minutes.

STEP 12: Remove the contents of the microwells by flicking the liquid into a suitable waste container and blotting off the residual solution. **Rinse each microwell 5 times with 200 µl of 1X Rinse Buffer**, flicking the liquid and blotting in between each wash.
Add 100 µl of Chromogen/Substrate Mixture (see **Preparation of Reagents**) to each well and incubate **without shaking** for 15 minutes in the dark. Positive samples turn blue.

STEP 13: Stop the color reaction by adding **100 µl of Stop Solution (Vial 8)** to each well. Positive samples will turn from blue to yellow.
Quantify results by use of a microplate or strip reader set to 450 nm.

INTERPRETATION OF RESULTS

- A positive result appears as a blue color which develops after addition of the Chromogen/Substrate Mixture. The blue color changes to yellow upon addition of the Stop Solution.
- The Hepatitis B Positive Control (Vial 9), serving as a control for only the microplate assay procedure, should give an OD of at least 0.5 (usually ≥ 1.5 OD units). The HBV DNA Preparation Control (Vial 10), serving to control both the preparative procedures and the assay procedures, should give an OD of at least 0.5 as well (usually 1.0-1.5 OD units).
- Each laboratory is advised to establish a negative cutoff OD value using known HBV DNA-negative specimens.
- Serum Specimen Titration Standards (ENZO Cat. No. 46354) can be used to quantitate results with patient specimens in the range of about 5 to 100 picograms of HBV DNA.
- Failure to obtain a positive OD value for a patient's specimen does not rule out the possibility of the presence of HBV DNA sequences and therefore, Hepatitis B Virus.
- If dilutions of a specimen are to be assayed, dilutions should be made in HBV DNA-negative serum.

For Technical Assistance call ENZO:
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